

(2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing a first gene expression pattern obtained from the cell of the first culture system with a second gene expression pattern expressed by a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a gene specific to the first gene expression pattern; and

(3) detecting the presence or absence of a gene exhibiting a specific expression to a first gene expression pattern, thereby detecting the endocrine disrupting action of the test substance.

2. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

A1 (1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

(b) culturing the cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) determining the presence or absence of an endocrine disrupting action of the test substance by obtaining a first gene expression pattern obtained from the cell in the first culture system and a third gene expression pattern obtained from the cell in the third culture system, comparing the first gene expression pattern with the third gene expression pattern and a second expression pattern expressed by a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a gene specific to the first gene expression pattern; and

(3) detecting the presence or absence of a gene exhibiting specific expression to the first gene expression pattern, thereby detecting the endocrine disrupting action of the test substance.

3. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

(b) culturing the cell a having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present;

M (2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing a first gene expression pattern obtained from the cell of the first culture system with a third gene expression pattern obtained from the cell in the third culture system, thereby detecting a gene specific to the first gene expression pattern; and

(3) detecting the presence or absence of a gene exhibiting specific expression to the first gene expression pattern, thereby detecting the endocrine disrupting action of the test substance.

4. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

(b) culturing the cell having a sensitivity to the endocrine hormone in a second culture system in which the endocrine hormone is present and the test substance is absent;
and

(c) culturing the cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing a first gene expression pattern obtained from the cell in the first culture system with a second gene expression pattern obtained from the cell in the second culture system and the third gene expression pattern obtained from the cell in the third culture system, thereby detecting a gene specific to the first gene expression pattern; and

(3) detecting the presence or absence of a gene exhibiting specific expression to the first gene expression pattern, thereby detecting the endocrine disrupting action of the test substance.

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6. (Amended) The method according to claim 1, wherein said endocrine hormone is triiodothyronine and said cell having a sensitivity to the endocrine hormone is Neuro2a.

A2
7. (Amended) The method according to claim 1, wherein comparison of the gene expression patterns are made by comparing bands obtained by subjecting a gene group contained in each of the gene expression patterns to electrophoretic separation.

8. (Amended) The method of detecting an endocrine disrupting action of a test substance according to claim 1, comprising

- (a) recovering RNAs from each of the culture systems of (1);
- (b) subjecting the RNAs recovered in the step (a) to reverse transcription;
- (c) amplifying reverse transcription products obtained in (b) by PCR; and
- (d) subjecting PCR products obtained in step (c) to electrophoresis, comparing electrophoretic patterns of the bands obtained, thereby detecting a band specific to the first gene expression pattern.

9. (Amended) The method according to claim 1, wherein said gene expression patterns are compared by hybridizing gene groups contained in each of the gene expression patterns with each other, and subtracting unhybridized genes.

10. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) determining the presence or absence of an endocrine disrupting action of the test substance by

(a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

AI (b) culturing a cell having a sensitivity to the endocrine hormone in a second culture system in which the endocrine hormone is present and the test substance is absent;

(c) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(d) culturing a cell having a sensitivity to the endocrine hormone in a fourth culture system in which both the endocrine hormone and the test substance are absent;

(2) (a) isolating RNA from the first culture system and preparing a first cDNA based on the RNA;

(b) isolating a second RNA from the second culture system;

(c) isolating a third RNA from the third culture system; and

(d) isolating RNA from the fourth culture system and preparing a fourth cDNA based on the RNA;

(3) (a) hybridizing the first cDNA and the second RNA and recovering unhybridized cDNA;

(b) hybridizing the third RNA and the fourth cDNA, recovering unhybridized RNA; and

(4) hybridizing the cDNA obtained in (a) of (3) and the RNA obtained in (b) of (3); and recovering unhybridized RNA, thereby detecting a gene specific to an endocrine disrupting action; and

A1 (5) detecting a specific gene to the endocrine disrupting action, thereby detecting the endocrine disrupting action of the test substance.

14. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) culturing a cell having a sensitivity to an endocrine hormone in a first culture system with the endocrine hormone and the test substance;

A2 (2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing a first glycoprotein pattern obtained from the cell of the first culture system with a second glycoprotein pattern expressed by a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a glycoprotein specific to the first glycoprotein pattern; and

(3) determining the presence and absence of a glycoprotein exhibiting specific expression to the first glycoprotein expression pattern, thereby detecting the endocrine disrupting action of the test substance.

15. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system with the endocrine hormone and the test substance;

(b) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) determining the presence or absence of endocrine disrupting action of the test substance by

obtaining a first glycoprotein pattern obtained from the cell of the first culture system and a third glycoprotein pattern obtained from the cell of the third culture system, and

comparing the first glycoprotein pattern with the third glycoprotein pattern and a second glycoprotein pattern obtained from a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a glycoprotein specific to the first glycoprotein pattern; and

AZ (3) determining the presence and absence of a glycoprotein exhibiting specific expression to the first glycoprotein expression pattern, thereby detecting the endocrine disrupting action of the test substance.

16. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

(b) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present; and

(2) determining the presence or absence of endocrine disrupting action of the test substance by comparing the first glycoprotein pattern obtained from the cell of the first

culture system with the third glycoprotein pattern obtained from the cell of the third culture system, thereby detecting a glycoprotein specific to the first glycoprotein pattern; and

(3) determining the presence and absence of a glycoprotein exhibiting specific expression to the first glycoprotein expression pattern, thereby detecting the endocrine disrupting action of the test substance.

17. (Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) (a) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present;

A2 (b) culturing a cell having a sensitivity to the endocrine hormone in a second culture system in which the endocrine hormone is present and the test substance is absent; and

(c) culturing a cell having a sensitivity to the endocrine hormone in a third culture system in which the endocrine hormone is absent and the test substance is present;

(2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing first, second and third glycoprotein patterns obtained respectively from the cells of the first, second, third culture systems, thereby detecting a glycoprotein specific to the first glycoprotein pattern; and

(3) determining the presence and absence of a glycoprotein exhibiting specific expression to the first glycoprotein expression pattern, thereby detecting the endocrine disrupting action of the test substance.

18. (Amended) The method according to claim 14, wherein said endocrine hormone is selected from the group consisting of estrogen, estradiol, progesterone, androgen, testosterone, androsterone, cortisol, aldosterone, corticosterone, cortison, triiodothyronine,

and thyroxine; and said cell having a sensitivity to the endocrine disrupting action is selected from the group consisting of Neuro2a, S-20Y, MCF7, TM3, TM4 and 15P-1.

19. (Amended) The method according to claim 14, wherein said endocrine hormone is triiodothyronine and said cell having a sensitivity to the endocrine hormone is Neuro2a.

20. (Amended) The method according to claim 14, wherein the glycoprotein specific to the first culture system is performed by a method comprising:

(a) extracting proteins biosynthesized by a cell contained in each of culture systems of (1);

(b) recovering a glycoprotein by binding a substance for specifically binding to a polysaccharide chain contained in the proteins extracted in (a) to the polysaccharide chain;

(c) recovering a protein contained in the glycoprotein by cutting off the polysaccharide chain; subjecting the protein to electrophoresis, thereby obtaining a glycoprotein pattern for each of the culture systems; and

(d) comparing glycoprotein patterns to each other.

Please add new Claims 24-28 as follows:

-24. (New) A method of detecting an endocrine disrupting action of a test substance, comprising:

(1) culturing a cell having a sensitivity to an endocrine hormone in a first culture system in which the endocrine hormone and the test substance are present, wherein said endocrine hormone is selected from the group consisting of a female hormone, male hormone, adrenal cortex hormone, and an amino acid derivative hormone;

(2) determining the presence or absence of an endocrine disrupting action of the test substance by comparing a first gene expression pattern obtained from the cell of the first

culture system with a second gene expression pattern expressed by a cell having a sensitivity to the endocrine hormone in the presence of the endocrine hormone, thereby detecting a gene specific to the first gene expression pattern; and

(3) determining the presence and absence of a glycoprotein exhibiting specific expression to the first glycoprotein expression pattern, thereby detecting the endocrine disrupting action of the test substance.

25. (New) The method according to Claim 24, wherein said cell having a sensitivity to the endocrine disrupting action is selected from the group consisting of Neuro2a, S-20Y, MCF7, TM3, TM4 and 15P-1.

A3 26 (New) The method according to Claim 24, wherein comparison of the gene expression patterns is made by comparing bands obtained by subjecting a gene group contained in each of said gene expression patterns to an electrophoretic separation.

27. (New) The method of detecting an endocrine disrupting action of a test substance according to Claim 24, comprising:

(a) recovering RNAs from each of the culture systems of (1);
(b) subjecting the RNAs recovered in the step (a) to reverse transcription;
(c) amplifying reverse transcription products obtained in (b) by PCR; and
(d) subjecting PCR products obtained in step (c) to electrophoresis, comparing the electrophoretic patterns of bands obtained, thereby detecting a band specific to a first gene expression pattern.

28. (New) The method according to Claim 24, wherein said gene expression patterns are compared by hybridizing gene groups contained in each of the gene expression patterns with each other, and subtracting unhybridized genes.